high dynamic binding capacity and selectivity for biomolecules. There further exists a need for a membrane that has low non-specific binding or low binding that results from hydrophobic interactions. There further exists a need for a membrane that can withstand high fluid flow velocities. There further exists a need for a membrane that involves preparation chemistries and/or processes that are not cumbersome to practice.

These advantages of the present invention, as well as additional inventive features, will be apparent from the description of the invention provided herein.

U.S. Patent 5,021,160 discloses copolymers synthesized from 2-acrylamido-2-methyl-1-propane sulfonic acid and either N-(isobutoxymethyl) acrylamide or 2-hydroxyethyl methacrylate and a process for preparing anionic charge modified microporous filtration membranes.

WO 98/17377 discloses charged membranes comprising a porous substrate and a cross-linked polyelectrolyte or hydrogel located in the pores of the substrate.

European Patent Application No. 0 474 617 A1 discloses a surface modified support membrane wherein the support membrane has a layer of hydrogel deposited on the surface thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts the breakthrough curve for lysozyme obtained on an embodiment membrane of the present invention. The x-axis represents the filtration time, and the y-axis represents the absorbance of the filtrate at 280 nm and is indicative of the concentration of the protein. See Example 2 for additional details.

Fig. 2 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present invention. The x-axis and y-axis are as described in Fig. 1. See Example 3 for additional details.

Fig. 3 depicts the breakthrough curve for lysozyme obtained on another embodiment membrane of the present

invention. The x-axis and y-axis are as described in Fig. 1. See Example 4 for additional details.

BRIEF SUMMARY OF THE INVENTION

Many of the foregoing needs have been fulfilled by the present invention which provides a negatively charged microporous membrane comprising a porous substrate and a crosslinked coating having negatively charged groups. In a preferred embodiment, the membrane can be prepared from a polymerized composition comprising an unsaturated monomer having an anionic group, at least one or more N-

polymerization is carried out for a period of from about 16 hours to about 24 hours. The viscosity of the solution is typically below about 2000 cps (2 Pa.s), e.g., preferably from about 50 cps (0.05 Pa.s) to about 500 cps (0.5 Pa.s), and more preferably from about 100 cps (0.1 Pa.s) to about 500 cps (0.5 Pa.s). According to certain embodiments, the viscosity is from about 100 cps (0.1 Pa.s) to about 250 cps (0.25 Pa.s).

The polymerization solution can contain the anionic acrylic monomer (A), the crosslinking agent (B), and the non-ionic hydrophilic monomer (C) in a suitable ratio. The percentage of each monomer (A, B, or C) can be from about 0.1 to 30% by weight, preferably from about 0.1 to 20% by weight.

It is believed that the crosslinked coating comprises amide-ester crosslinks that form as a result of the reaction of the nonionic hydrophilic monomer with the crosslinking agent. For example, these bonds form as a result of the reaction of the hydroxyl groups in the hydroxyalkyl acrylate with the N-(isobutoxymethyl)- acrylamide. In addition, amide-amide crosslinks also form as a result of the reaction between two N-(isobutoxymethyl)acrylamide monomers. For example, the amide-ester crosslink can have the formula:

$$-C(=O)O-R-NH-C(=O),$$

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-CH_2-CH_2-O-CH_2-$. The amide-amide crosslink can have the formula:

$$-C(=O)NH-R-NH-C(=O),$$

wherein R is divalent radical, preferably an alkoxyalkyl radical, and more preferably $-CH_2-O-CH_2-$.

The coating solution contains the anionic polymer prepared as above and, optionally, a polysaccharide, preferably a dextran. The anionic polymer and the polysaccharide can be present in the coating solution in the ratio of from about 100:1 to about 1:100, preferably from about 10:1 to about 1:10, and more preferably from about 5:1 to about 1:5.

The coating solution contains the anionic polymer and, optionally dextran, in an amount of from about 0.01% to about

for example, the membrane preferably has a water flow rate above 5 mL/min/cm^2, and preferably above 10 mL/min/cm^2, e.g., from about 20 mL/min/cm^2 to about 160 mL/min/cm^2, and preferably from about 25 mL/min/cm^2 to about 100 mL/min/cm^2 at 24 inch Hg. The membrane is robust and can withstand high treatment fluid flow rates. Thus, the membrane can be subjected to flow rates up to 10 cm/min, for example, from about 1 cm/min to 10 cm/min at 10 psi (68.9 kPa). The membrane has an open water bubble point of below about 70 psi (482 kPa), e.g., from about 2.5 psi (9.39 kPa) to about 70 psi (482 kPa), and preferably from about 5 psi (34.47 kPa) to about 50 psi (344.7 kPa).

The membrane of the present invention has a high charge density. The charge density of the membrane can be measured by methods known to those of ordinary skill in the art. For example, the charge density can be measured by the membrane's ability to bind a positively charged dye. Illustratively, the membrane has a Methylene Blue dye binding capacity of at least about 10 mL, e.g., from about 10 mL to about 1000 mL, and preferably from about 100 mL to about 800 mL, when tested with a 10 ppm dye solution in water. Methylene Blue is a positively charged dye. The dye binding capacity is measured by filtering under a 24 inch Hg negative pressure, a 10 ppm by weight solution, pH 6.6, of Methylene Blue dye in a membrane disc of 25 mm diameter, and monitoring the volume of the filtrate until a trace of the dye begins to appear in the filtrate.

The membrane of the present invention has a high specific protein binding capacity. The membrane has a lysozyme specific binding capacity of above 10 mg/mL, e.g., from about 10 mg/mL to about 130 mg/mL and preferably from about 25 mg/mL to about 120 mg/mL. The specific binding capacity can be determined by the following illustrative method. A fluid containing a lysozyme protein in 10 mM MES buffer, pH 5.5, is filtered by passing through a membrane at 1 cm/min and the concentration of the protein in the filtrate is measured as a function of time. The concentration of the protein can be

determined spectrophotometrically, e.g., by measuring the

stack can be eluted under a gradient - 7 ml from 10 mM MES buffer at a pH of 5.5 to 1M NaCl-10 mM MES buffer at a pH of 5.5. The flow rate can be 4 ml/min. Cytochrome C elutes first, followed by lysozyme.

The following examples further illustrate the present invention but should not be construed in any way limiting the scope of the invention.

EXAMPLE 1

This Example illustrates a method of preparing a polymer composition for preparing an embodiment of the negatively charged membrane of the present invention.

2-Acrylamido-2-methyl-1-propanesulfonic acid, N-(isobutoxymethyl)acrylamide, and hydroxypropyl methacrylate were combined in a weight ratio of 8.0:2.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 12% by weight. Ammonium persulfate was used as the initiator at 0.3% by weight of the solution. The polymerization was carried out for a period of about 10-15 hours at ambient temperature (20-25°C). The resulting solution had a viscosity of 166 cps (0.166 Pa.s).

EXAMPLE 2

This Example illustrates a method for preparing an embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

A coating solution was prepared by mixing the polymerization solution described in Example 1 and a water solution of dextran, molecular weight 148 K, so that the resulting solution contains polymer and dextran in the weight ratio of 3:1.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 μm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI

first 10 minutes of the treatment confirmed that the membrane did not leak. The nearly vertical slope indicates that the membrane was capable of providing high resolution.

EXAMPLE 4

This Example illustrates a method for preparing another embodiment of the negatively charged membrane of the present invention. This Example further illustrates the properties of that embodiment.

2-Acrylamidoglycolic acid, 2-carboxyethyl acrylate, N-(isobutoxymethyl)acrylamide, N-(hydroxymethyl)-acrylamide, and hydroxypropyl acrylate were combined in a weight ratio of 5.0:5.0:3.0:1.5:1.5 in a methanol-water medium to obtain a polymerization solution having a solids content of 16% by weight. Ammonium persulfate was used as the initiator at 0.4% by weight of the solution. The polymerization was carried out for a period of about 16-24 hours at ambient temperature. The resulting solution had a viscosity of 116 cps (0.116 Pa.s). A coating solution was prepared by mixing the polymerization solution and a water solution of dextran, molecular weight 148 K, so that the resulting solution contained 4% polymer and 1.33% dextran by weight.

A hydrophilic microporous polyethersulfone substrate having a pore size of about 0.8 µm was coated with the above coating solution. The coated substrate was cured in an oven at 100-110°C for 1 hour, followed by washing it in boiling DI water for 1 hour. The resulting membrane was dried in an oven to obtain another embodiment of the present invention.

The membrane obtained above was tested with a solution containing lysozyme. The solution was contained 213.6 µg per ml of 10 mM MES buffer at pH 5.5. The treatment fluid flow rate was 4 ml/min. Two membrane discs of 25 mm diameter were stacked together. The breakthrough curve obtained is set forth in Fig. 3. The membrane had a lysozyme binding capacity of 45 mg/ml. The relatively flat curve obtained during the first 10 minutes of the treatment confirmed that the membrane